or storage of the particles). In some embodiments, the cryoprotectant includes one or more sugars (e.g., one or more dissacharides (e.g., trehalose, melizitose, raffinose)) and/or one or more poly-alcohols (e.g., mannitol, sorbitol).

[0140] Lyophilized particles can be prepared as desired. Typically, the particles are prepared using a cryoprotectant and chilled hydrophobic surface. Typically, compounds of the lyophilized particles are combined with a solvent (e.g., water) to make a solution, which is then placed (e.g., in discrete aliquots (e.g., drops) such as by pipette) onto a chilled hydrophobic surface (e.g., a diamond film or a polytetrafluorethylene surface). In general, the temperature of the surface is reduced to near the temperature of liquid nitrogen (e.g., about −150° F. or less, about −200° F. or less, about −275° F. or less). The solution freezes as discrete particles. The frozen particles are subjected to a vacuum while still frozen for a pressure and time sufficient to remove the solvent (e.g., by sublimation) from the pellets.

[0141] For example, a solution for preparing particles can be prepared by combining a cryoprotectant (e.g., 6 grams of trehalose), a plurality of particles modified with ligands (e.g., about 2 milliliters of a suspension of carboxylate modified particles with poly-D-lysine ligands), a protease (e.g., 400 milligrams of pronase), an RNAase (e.g., 30 milligrams of RNAseA (activity of 120 U per milligram), an enzyme that digests peptidoglycan (e.g., ReadyLyse (e.g., 160 microliters of a 30000 U per microliter solution of ReadyLyse)), a cell specific enzyme (e.g., mutanolysin (e.g., 200 microliters of a 50 U per microliter solution of mutanolysin), and a solvent (e.g., water) to make about 20 milliliters. About 1,000 aliquots of about 20 microliters each of this solution are frozen and desolvated as described above to make 1,000 pellets. When reconstituted, the pellets are typically used to make a total of about 200 milliliters of solution.

[0142] In general, the concentrations of the compounds in the solution from which the particles are made is higher than when reconstituted in the microfluidic device. Typically, the ratio of the solution concentration to the reconstituted concentration is at least about 3 (e.g., at least about 4.5). In some embodiments, the ratio is about 6.

Operation

[0143] In an exemplary embodiment, cartridge **200** may be operated as shown in FIGS. **4** and **15-27**, and as described as follows. It is to be understood that these figures depict an exemplary embodiment and that other embodiments are within the scope of the present invention, for example the exemplary operation described in FIGS. **6-17** of U.S. provisional application Ser. No. 60/726,066, filed Oct. 11, 2005, and incorporated herein by reference in its entirety.

[0144] Prior to sample processing, valves of component **201** are configured in the open state, and gates of component **201** are configured in the closed state.

[0145] Approximately 1.5 milliliters of clinical sample **600**, in fluid form, is input into bulk lysis chamber **264** through sample inlet **202**. For example, sample can be introduced with a syringe having a fitting complementary to a luer on sample inlet **202**. In other embodiments, the amount of sample introduced is about 500 microliters or less (e.g., about 250 microliters or less, about 100 microliters or less, about 50 microliters or less, about 25 microliters or less, about 10 microliters or less). In some embodiments, the amount of sample is about 2 milliliters or less (e.g., 1.5 milliliters or less).

[0146] An excess amount of air (about 1-3 ml and typically 2.5 ml) of air is also injected into the sealed bulk lysis chamber, through sample inlet **202** preferably at the same time that the sample is injected. The air above the fluid sample is under compression during this stage until the pressure is released later on.

[0147] The liquid sample dissolves the bulk lysis reagent pellets and capture reagent pellets in the lysis chamber **264**, if present. Reconstituted lysing reagents (e.g., ReadyLyse, mutanolysin) begin to lyse cells of the sample releasing polynucleotides. Other reagents (e.g., protease enzymes such as pronase) begin to reduce or denature inhibitors (e.g., proteins) within the sample. Polynucleotides from the sample begin to associate with (e.g., bind to) ligands of particles released from the pellets.

[0148] The cartridge is placed in the cartridge receiving element of a system such as system **10**, FIG. **1**, either after the sample is introduced of before. The user instructs the system to proceed with sample preparation, say by delivering appropriate instructions through a user interface **32**. In preferred embodiments, the system begins sample preparation automatically after the cartridge receiving element has accepted a cartridge and has communicated its acceptance to a controller.

[0149] The sample in the bulk lysis chamber is heated up to a temperature sufficient to initiate chemical lysis of the cells. Lysing of cells may occur by application of heat alone, or by a combination of heat and lysis reagents, as described herein. The chamber is typically at a temperature of about 50° C. or less (e.g., 30° C. or less) during introduction of the sample. Typically, the sample within chamber **264** is heated to a temperature in the range 60-80° C. (e.g., to at least about 65° C., to at least about 70° C.) for a short period of time, preferably 5-10 minutes, (e.g., for about 15 minutes or less, about 10 minutes or less, about 7 minutes or less) while lysing occurs.

[0150] In some embodiments, a heat lamp in close proximity to the bulk lysis chamber, heats the sample. In other embodiments, optical energy is used at least in part to heat contents of lysing chamber **264**. For example, the operating system used to operate device **300** can include a light source (e.g., a lamp primarily emitting light in the infrared) disposed in thermal and optical contact with chamber **264**. An especially preferred manner of heating is by contact heating, such as by direct contact of a heating element with upper surface **266** of the lysis chamber, as accomplished by exemplary system **10** of FIG. **1**. Chamber **264** preferably includes a temperature sensor used to monitor the temperature of the sample within chamber **264**. The heat output of the heat source is increased or decreased based on the temperature determined with sensor.

[0151] The bulk lysis reagents contain a cocktail of reagents that chew up the cell walls of the target cells, chew up PCR inhibiting proteins, lipids, etc., and also have DNA (or RNA) affinity beads (~10 micron in median diameter) that capture DNA (or RNA) present in the sample. This process typically takes between about 1 and about 5 minutes.

[0152] Polynucleotides of the sample contacting the affinity beads are preferentially retained as compared to liquid of